Smithsonian Tropical Research Institute

Minera Panamá

PROYECTO MINA DE COBRE PANAMÁ

ESTUDIO DE IMPACTO AMBIENTAL (EsIA)

CATEGORIA III

INFORME TRIMESTRAL DEL PROYECTO DE CONSERVACIÓN EX SITU DE ESPECIES DE INTÉRES DE ANFIBIOS

Compromisos de EsIA Aplicables	Periodo reportado	Elaborado por	Fecha de Emisión del Reporte
13202, 13207 13208, 13212 13221, 13223 13232, 13233	**Noviembre-Enero**	**INSTITUTO SMITSONIAN DE INVESTIGACIONES TROPICALES**	**21/01/2016**

INFORME TRIMESTRAL DEL PROYECTO

"PROPUESTA PARA LA EXTENSIÓN DE LA CONSERVACIÓN EX-SITU DE RANAS DEL ÁREA DE DONOSO POR DOS AÑOS"

Período: Noviembre 2015-Enero 2016

Por: Roberto Ibáñez D., Proyecto de Rescate y Conservación de Anfibios de Panamá, Smithsonian Tropical Research Institute (STRI)

Fecha: 21 de enero de 2016

Introducción

El 1 de junio de 2015, Minera Panamá S.A. (MPSA) y el Instituto Smithsonian de Investigaciones Tropicales (STRI) firmaron un acuerdo con el fin de extender el proyecto de conservación ex situ de anuros del área de Donoso por 2 años. Con este acuerdo se realizarán esfuerzos de conservación e investigación que contribuirán al manejo y preservación de los anfibios de Donoso, enfocándose en las especies de interés (EdI). Este proyecto es coordinado por el Proyecto de Rescate y Conservación de Anfibios de Panamá (PARC) y es llevado a cabo en dos instalaciones para la conservación de este amenazado grupo de vertebrados: (1) el Centro de Conservación de Anfibios de El Valle (EVACC), y (2) el Centro de Investigación y Conservación de Anfibios de Gamboa (Gamboa-ARCC). En este informe se presentan los avances, según los objetivos acordados de este proyecto, realizados durante el período Noviembre de 2015-Enero de 2016.

Objetivo 1. – Mejorar los procedimientos y tácticas para el manejo de los anfibios, desarrollando mejores prácticas para la conservación ex situ de anfibios en riesgo.

Basados en los resultados del taller *"Conservación de Anfibios de Panamá: Evaluación de Especies Amenazadas para Programas Ex situ y la Lista Roja de la UICN*", que realizamos el 23 de octubre de 2015, preparamos una lista de las especies evaluadas para la Unión Internacional para la Conservación de la Naturaleza (UICN). Esta lista presenta las categorías actualizadas que sugerimos para cada una de las 40 especies de anfibios de Panamá que evaluamos, según los criterios de la UICN (Cuadro 1). El oficial de programa, de la autoridad de la lista roja de anfibios de la UICN SSC, la verificará y, posteriormente, realizará las

modificaciones pertinentes a la información disponible en línea sobre estas especies (http://www.iucnredlist.org/initiatives/amphibians).

Actualización de la Lista Roja sugerida durante el taller	
Especie	**Categoría y criterios**
Agalychnis lemur	CR (sin cambio)
Andinobates geminisae	CR A3ce; B1ab(i,ii,iii,iv,v)
Anomaglossus astralogaster	DD
Anomaglossus isthminus	EN A2ce; B1ab(iv)
Anotheca spinosa	EN A4ce; B1ab(i,ii,iii,iv)
Atelopus certus	CR A3ce
Atelopus chiriquiensis	EX
Atelopus glyphus	CR A4ce
Atelopus limosus	CR A4ce
Atelopus varius	CR A2ace
Atelopus zeteki	CR A2ace
Bolitoglossa chucantiensis	DD
Bolitoglossa jugivagans	DD
Bolitoglossa pygmea	NT
Bolitoglossa robinsoni	NT
Colostethus panamansis	EN A4ce
Craugastor emcelae	CR
Craugastor evanesco	CR A4ace
Craugastor obesus	CR
Craugastor punctariolus	CR
Craugastor rhycobatrachus	CR
Diasporus citrinobapheus	LC
Diasporus igneus	CR A3c; B1ab(i,ii,iii,iv)
Ecnomiohyla bailarina	VU A3ce; B1ab(i,ii,iii,iv)
Ecnomiohyla rabborum	CR
Ecnomiohyla veraguensis	EN A3ce
Gastrotheca cornuta	CR A2ace; B1ab(i,ii,iii,iv,v)
Hemiphractus fasciatus	EN A4ce; B1ab(i,ii,iii,iv,v)
Hyloscirtus colymba	CR (sin cambio)
Hyloscirtus palmeri	VU A4ce; B1ab(iii)
Incilius karenlipsae	DD
Incilius majordomus	EX
Isthmohyla calypsa	CR
Leptodactylus insularum	LC
Oedipina fortunensis	NT
Oophaga speciosa	CR
Oophaga vicentei	VU A4cde
Pristimantis adnus	NT
Rhinella centralis	LC
Strabomantis bufoniformis	CR A4ace

Cuadro 1. Actualización sugerida para la Lista Roja de la UICN de las especies de anfibios panameños evaluadas durante el taller. EX = Extinta, CR = En Peligro Crítico, EN = En Peligro, VU = Vulnerable, NT = Casi Amenazada, LC = Preocupación Menor, DD = Datos Insuficientes.

Adicionalmente, se publicó en línea el artículo *"Evaluating the probability of avoiding disease-related extinctions of Panamanian amphibians through captive breeding programs"* (= "*Evaluación de la probabilidad para evitar extinciones asociadas a enfermedades en anfibios panameños, a través de programas de cría en cautiverio"*) en la revista Animal Conservation (Anexo 1). Esta publicación permite refinar las prioridades para los programas de conservación ex situ de anfibios en Panamá e identificar las acciones prioritarias para su conservación con un entendimiento más claro de las probabilidades de éxito. Los resultados sugieren que la cría en cautiverio puede mejorar las posibilidades para evitar la extinción de especies que han declinado severamente o que probablemente declinarán debido a la quitriomicosis, entre estas especies están *Agalychnis lemur*, *Anotheca spinosa*, *Atelopus certus*, *A. glyphus*, *A. limosus*, *A. varius*, *A. zeteki*, *Gastrotheca cornuta* y *Hemiphractus fasciatus*.

Objetivo 2. – Llevar a cabo expediciones para recolectar especies de anfibios en riesgo en el área de Donoso.

Durante este período, se llevó a cabo una expedición, desde el 10 hasta el 19 de diciembre de 2015, con la intención de buscar y colectar individuos de tres Especies de Interés (EdI): *Atelopus varius, Craugastor evanesco* y *Gastrotheca cornuta*, dentro del área de desarrollo del Proyecto Mina de Cobre Panamá, distrito de Donoso, provincia de Colón. En esta expedición, Jorge Guerrel, Rigoberto Díaz y Edgardo Griffith recolectaron individuos fundadores para el programa de conservación ex situ. Recolectaron 1 hembra juvenil de *A. varius* y 1 juvenil y 1 macho adulto de *C. evanesco.* Estos individuos fueron depositados en el EVACC.

***Atelopus varius* macho, Quebrada Valle Grande, área de desarrollo del Proyecto Mina de Cobre Panamá.**

Los individuos antes mencionados resultaron negativos al *B. dendrobatidis* al momento de ser colectarlos en el campo, según el análisis de qPCR realizado de los hisopos de campo. Actualmente, éstos se encuentran en cuarentena. Al individuo de *A. varius* no se le está

aplicando el tratamiento preventivo con Itraconazol, ya que es posible que este tratamiento tenga algún efecto negativo en su supervivencia; mientras que, a los individuos de *C. evanesco* se les está aplicando dicho tratamiento. Estamos dándole seguimiento a cada uno de los individuos en cuarentena, a través de hisopados de la piel tomados cada 10 días y pruebas de qPCR para detectar la presencia del *B. dendrobatidis*. De esta forma, es posible tratar con Itraconazol sólo a aquellos individuos infectados por este hongo; evitando así, cualquier efecto secundario indeseado del medicamento.

Objetivo 3. – Completar y mantener instalaciones para la cría y reproducción de poblaciones en cautividad de las especies de anfibios de interés de Donoso (fondos para dos años adicionales).

Se reemplazó el aire acondicionado de la exhibición del EVACC. También, hemos realizado algunas reparaciones menores de los aires acondicionados de los contenedores del Centro de Investigación y Conservación de Anfibios de Gamboa (Gamboa-ARCC).

La producción de invertebrados se ha mantenido al nivel requerido, tanto en EVACC como Gamboa-ARCC, donde se producen principalmente grillos, dos especies de moscas de la frutas, colémbolos y cucarachas para alimentar a las poblaciones de anfibios en cautiverio.

Los números de individuos de las poblaciones en cautiverio de las especies de anuros, procedentes del área de Donoso, que mantenemos actualmente en nuestras instalaciones son:

Especie	Machos	Hembras	Juveniles	Ranas totales	Reproducción en cautiverio	Renacuajos	Instalación
Andinobates geminisae	15	18	3	36	+	3	Gamboa-ARCC
Atelopus varius	31	14	6	51	+	>200	EVACC y Gamboa-ARCC
Craugastor evanesco	25	19	3	44	+	-	EVACC
Ecnomiohyla miliaria	2	0	0	2	-	0	EVACC
E. veraguensis	2	0	0	2	-	0	EVACC
Incilius coniferus	1	1	1	3	-	0	EVACC (exhibición)
Gastrotheca cornuta	1	0	0	1	-	-	EVACC
Oophaga vicentei	24	24	6	54	+	~32	Gamboa-ARCC y EVACC
Total	**101**	**76**	**16**	**193**		**>235**	Gamboa-ARCC y EVACC

Nota: (-)*Craugastor evanesco* y *Gastrotheca cornuta* no presentan etapa de renacuajo libre.

Al 24 de enero de 2016, manteníamos 193 ranas de 8 especies de anuros del área de Donoso. Entre las especies que se mantienen en cautiverio están las cuatro Especies de Interés (EdI) del Proyecto Mina de Cobre Panamá: *Andinobates geminisae, Atelopus varius, Craugastor evanesco* y *Gastrotheca cornuta*. Adicionalmente, el proyecto PARC mantiene especies endémicas de distribución restringida: *Ecnomiohyla veraguensis* y *Oophaga vicentei*, que se encuentran en el área de Donoso y las cuales pueden ser consideradas como especies en peligro y vulnerable, respectivamente. Otras especies, también, provenientes del área de desarrollo del Proyecto Mina de Cobre Panamá, como *Ecnomiohyla miliaria* e *Incilius coniferus*, se tienen con el propósito de estudiarlas por ser especies raras o para exhibición.

Prácticamente tenemos suficientes individuos fundadores para tres de estas especies: *Andinobates geminisae, Craugastor evanesco* y *Oophaga vicentei,* aunque sería recomendable obtener 2 hembras de *C. evanesco* y 7-9 individuos de *A. geminisae* más. Requerimos recolectar 6-8 hembras de *Atelopus varius*, para completar el número inicial recomendado de individuos fundadores para estas dos especies. En el caso de *Gastrotheca cornuta*, a pesar de los esfuerzos de colecta realizados en el área, no ha sido posible encontrar más de un individuo, pero continuamos buscándolos.

Durante el período Noviembre-Enero, hubo varios eventos de reproducción de las especies *Andinobates geminisae* y *Oophaga vicentei*, los cuales aún constituyen el inicio para establecer poblaciones estables en cautiverio del programa de conservación ex situ. Al final del período, estimamos que se tenían unos 35 renacuajos de estas dos especies, ya que no se pueden contar todos los renacuajos por encontrarse entre las axilas de las hojas de las plantas de bromelias. Tenemos más de 200 renacuajos de *Atelopus varius*, provenientes de una puesta reportada en el informe anterior. Además, durante el período actual, preparamos más tanques de reproducción para cruzar parejas de *Atelopus varius;* lo que ha dado como resultado, tres puestas de huevos (pero, dos de éstas resultaron infértiles) y dos parejas en amplexus al final de este período. En cuanto a reproducción, lo más interesante en el período actual, han sido las 2 puestas de huevos por dos parejas de *Craugastor evanesco* en nidos que ellos excavan en el sustrato. Es la primera vez que se observa el comportamiento de anidación en esta especie. Esperamos que los huevos se desarrollen normalmente.

Objetivo 4. - Estudio de los agentes patógenos de anfibios, hongos Batrachochytrium dendrobatidis y Fusarium cf. solani, en la región de Donoso.

En el transcurso del trabajo que hemos estado realizando en el área de Donoso, observamos una alta mortalidad en los individuos de *Atelopus varius* recolectados. El problema en estos individuos empieza con la aparición de pequeñas lesiones en la piel, la cuales se van agrandando en tamaño y, luego, aparece una especie de moho en su piel, que termina con la muerte del individuo. Las lesiones iniciales se han detectado en individuos recién llegados a nuestras instalaciones, pero se agravan durante el período de cuarentena. Exámenes de necropsia muestran que las ranas presentaban una fuerte dermatitis, aparentemente causada por una especie de hongo, *Fusarium cf. solani*, distinto al hongo quitridio *B. dendrobatidis*. No obstante, lógicamente, es necesario: (1) determinar la especie del hongo *Fusarium* de manera precisa, (2) probar que este hongo es el patógeno que causa la enfermedad y muerte en las ranas, y (3) diagnosticar la enfermedad a tiempo y tratar las ranas infectadas. Consecuentemente, requerimos realizar estudios de sistemática molecular sobre el hongo *Fusarium*, exámenes patológicos de las ranas, experimentos para establecer si es el agente causante de la enfermedad y realizar ensayos que permitan detectar el patógeno y curar a las ranas afectadas.

El 9 de diciembre iniciamos los primeros ensayos con sapitos túngara, *Engystomops pustulosus*, para establecer el hongo *Fusarium cf. solani* cumple con los postulados de Koch y realmente es el agente causante de infección y muerte en anuros. Además, pretendemos determinar si el tratamiento con Itraconazole hace a las ranas más susceptibles a enfermarse y fallecer por este hongo potencialmente oportunista. Estamos utilizando esta especie, ya que es una especie común y no es una especie amenazada como *Atelopus varius*. Según los resultados que se obtengan estos primeros experimentos, diseñaremos ensayos subsiguientes.

En cuanto a los cultivos de dos cepas de *Fusarium cf. solani*, originalmente aisladas de individuos de ranas de *Atelopus varius* del área de Donoso, que fueron enviados al Dr. Douglas

Woodhams de la University of Massachusetts Boston. El Dr. Woodhams y su equipo de trabajo han realizado ensayos de inhibición con una de estas cepas, inicialmente utilizando soluciones que varían en su pH y soluciones de cloruro de sodio, cloruro de benzalconio y povidona yodada. Además, están realizando ensayos de inhibición con otros compuestos (i.e., antibióticos y antimicóticos), los cuales están actualmente en curso o por iniciar.

El Dr. Kerry O'Donnell del National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture (NCAUR-ARS-USDA), recibió las cuatro cepas de *Fusarium cf. solani* que, también, fueron originalmente aisladas de individuos de ranas de *Atelopus varius* del área de Donoso. El Dr. O'Donnell, que es un especialista en este género de hongos, nos indicó que ya ha podido secuenciar el ADN de tres de estas cepas, lo cual ayudará a la identificación de las mismas.

En cuanto al hongo *B. dendrobatidis,* durante la expedición del 10-19 de diciembre de 2015, se obtuvieron muestras de la piel con hisopos de 63 anuros que fueron encontrados en el área de Donoso; en quebrada Valle Grande y el Vado. Los anuros que fueron muestreados incluyen individuos de las especies EdI colectadas e individuos de éstas y otras especies que fueron liberadas inmediatamente después de tomarles la muestra. Por el momento, solamente se han analizado las muestras de los anuros que fueron depositadas en el EVACC, las cuales resultaron *B. dendrobatidis* negativos. El resto de las muestras serán procesadas próximamente.

Anexo 1. Artículo *"Evaluating the probability of avoiding disease-related extinctions of Panamanian amphibians through captive breeding programs"* (= "*Evaluación de la probabilidad para evitar extinciones asociadas a enfermedades en anfibios panameños, a través de programas de cría en cautiverio"*).

Animal Conservation

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Animal Conservation. Print ISSN 1367-9430

Evaluating the probability of avoiding disease-related extinctions of Panamanian amphibians through captive breeding programs

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Keywords
extinction probability; Panama; amphibian; chytridiomycosis; *ex situ* conservation; captive breeding; expert survey; disease.

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Editor: John Ewen
Associate Editor: Tara Martin

Received 08 May 2015; accepted 03 November 2015

doi:10.1111/acv.12249

Abstract

Amphibians around the world are declining from threats that cannot currently be mitigated, making it impossible to safeguard some species in their natural habitats. Amphibians in the mountainous neotropics are one example where severe disease-related declines prompted calls for the establishment of captive assurance colonies to avoid extinctions. We surveyed experts in Panamanian amphibians to determine the probability of avoiding chytridiomycosis-related extinctions using captive breeding programs. We ranked Panamanian amphibian species by perceived susceptibility to chytridiomycosis, then calculated the likelihood of avoiding extinction as the product of three probabilities, which include (1) finding sufficient founder animals, (2) successfully breeding these species in captivity and (3) becoming extinct in the wild. The likelihood of finding enough animals to create a captive founding population was low for many rare species, especially for salamanders and caecilians. It was also low for frogs which were once regularly encountered, but have already disappeared including *Atelopus chiriquiensis*, *Craugastor emcelae*, *C. obesus*, *C. punctariolus*, *C. rhyacobatrachus*, *Ecnomiohyla rabborum*, *Isthmohyla calypsa* and *Oophaga speciosa*. Our results indicate that captive breeding could improve the odds of avoiding extinction for species that have severely declined or are likely to decline due to chytridiomycosis including *Atelopus certus*, *A. glyphus*, *A. limosus*, *A. varius*, *A. zeteki*, *Anotheca spinosa*, *Gastrotheca cornuta*, *Agalychnis lemur*

and *Hemiphractus fasciatus*. Priority species that experts predicted were highly susceptible to chytridiomycosis that might also benefit from *ex situ* management include *Craugastor tabasarae*, *C. azueroensis*, *C. evanesco*, *Strabomantis bufoniformis* and *Colostethus panamansis.* In spite of high levels of uncertainty, this expert assessment approach allowed us to refine our priorities for captive amphibian programs in Panama and identify priority conservation actions with a clearer understanding of the probability of success.

Introduction

Compared to other terrestrial vertebrates, amphibians are disproportionately threatened with extinction (Stuart *et al.*, 2004; Wake & Vredenburg, 2008) and receive much less conservation funding (Gratwicke, Lovejoy & Wildt, 2012*b*). Efficiently deploying limited resources and increasing existing capacity to conserve amphibians is imperative. Species-level conservation prioritization schemes typically evaluate level of IUCN-related endangerment (Master, 1991; Mace *et al.*, 2008), although additional criteria have been used including phylogenetic distinctiveness (Isaac *et al.*, 2007), types of conservation action (Carter *et al.*, 2000), costs and benefits (Weitzman, 1998) and probability of success (Joseph, Maloney & Possingham, 2009). However, a one-size fits all approach to setting priorities is unlikely to be useful because priorities of any program depend on specific goals and context.

Panama has 214 described amphibian species (AmphibiaWeb, 2015) that are ecologically and phylogenetically diverse, and about one third are listed by the IUCN Red List of Threatened Species as at risk of extinction. Since 1996, Panamanian amphibians have declined due to chytridiomycosis, a disease caused by an invasive fungus *Batrachochytrium dendrobatidis* (*Bd*) that has devastated naive amphibian communities (Lips, 1999; Crawford, Lips & Bermingham, 2010; Cheng *et al.*, 2011). Species declines and extirpations prompted an emergency response to create captive breeding programs (Lock, Mendelson & Gagliardo, 2006; Gagliardo *et al.*, 2008; Poole, 2008; PARC, 2010; ANAM, 2011; Zippel *et al.*, 2011). In 2000 Project Golden Frog was started to build captive assurance populations of *Atelopus zeteki* and *Atelopus varius* in the USA and after starting with a small founding population, the project now manages 1600 captive golden frogs in 50 zoos and aquaria (Poole, 2008). The last golden frog was seen in the wild in 2009, so this captive breeding project may have already avoided a *Bd*-related extinction, and an ambitious golden frog conservation plan was developed by multiple stakeholders to build the capacity, management, outreach, research and habitat goals that will allow us to pilot experimental reintroductions of this iconic species (Estrada *et al.*, 2014). A second effort to establish captive assurance colonies of more amphibian species began in 2006 involving two independent frog rescue response teams. One worked from El Copé to rescue frogs in the midst of the *Bd* epidemic and a second collected animals in the El Valle area 40 km ahead of the disease wave (Norris, 2007). Because the die-off was so severe, the strategy involved capturing individuals of all identified frog species, even though little was known about the captive husbandry requirements of most species. Over time, much was learned about husbandry (Gagliardo *et al.*, 2008), treatment of chytridiomycosis (Baitchman & Pessier, 2013) and specific causes of mortality including nutritional issues, polycystic kidney lesions, parasites, metabolic bone disease (Pessier *et al.*, 2014).

In 2009, the Panama Amphibian Rescue and Conservation (PARC) project began as an in-country effort to build additional capacity and manage captive assurance populations. Amphibians are held at two *ex situ* holding facilities, the El Valle Amphibian Conservation Center (EVACC) and the Gamboa Amphibian Research and Conservation Center (Gamboa ARCC). Besides improving husbandry, the goal of PARC was to establish self-sustaining, reproducing colonies, and to research methods to reduce the impact of *Bd* so that one day amphibians produced in captivity could be reintroduced to the wild (Gratwicke *et al.*, 2012*a*). The PARC project used an early tool developed by the IUCN's Amphibian Ark to prioritize species for captive breeding (AArk, 2009). This additive index considers extinction risk, phylogenetic significance, reversibility of threats, biological distinctiveness as well as cultural and scientific importance to produce a single numerical score ranking for priority. More recently, the process was refined to include animal availability and management capacity (AArk, 2009), but it does not explicitly evaluate the likelihood of avoiding extinction through captive breeding efforts.

The PARC project has a diverse collection of amphibians and there is a need to focus effort on those species where captive breeding efforts will have the highest likelihood of avoiding extinction. In reality, this is easier said than done because there remains little empirical data on susceptibility to chytridiomycosis, rates of decline, wild population sizes and captive husbandry at a species level. In these situations, expert opinion surveys are often the best way to systematically capture the needed information (Fazey *et al.*, 2006; Kuhnert, Martin & Griffiths, 2010; Raymond *et al.*, 2010). The objective for this study was to conduct an expert opinion survey to evaluate the perceived susceptibility of Panama's amphibians to chytridiomycosis and filter the list of species to determine the species that had or would likely experience *Bd*-related declines. For the susceptible species, we calculate the probability of avoiding extinction through captive breeding efforts as the product of three probabilities determined from expert responses (1) finding sufficient

founder animals, (2) successfully breeding these species in captivity and (3) becoming extinct in the wild.

1 *Finding sufficient founder animals*: The probability of finding enough wild males and females to form a viable captive population varies depending on whether the species is rare, difficult to find, or has already declined. One of the greatest risks to a species in a captive breeding program is genetic deterioration resulting from inbreeding depression (Frankham, Briscoe & Ballou, 2002). To minimize the potential loss of genetic heterozygosity, a population of adequate size is required to retain 90% of existing genetic diversity for the next 100 years (Frankham *et al.*, 2002). Recommended numbers of founders vary from species to species depending on age at sexual maturity and reproductive life span (Schad, 2008). At least 10 unrelated male–female pairs is an approximate guideline for the minimum numbers needed for amphibian conservation breeding programs, and given that the probability of breeding founders is not 100%, these numbers should be increased accordingly (Schad, 2008).
2 *Successfully breeding these species in captivity*: A few colorful Latin American species such as *Dendrobates auratus*, *Oophaga pumilio* and *Agalychnis callidryas* are popular pets or widely kept in zoos and aquaria. Husbandry tactics for these species are well known to hobbyists and the zoo community. With care and attention to detail, these species can be bred in captivity, and may be useful models for understanding the husbandry requirements of related amphibians. However, the natural history of many tropical amphibian species is virtually unknown, and some species do not adapt well to captivity because we cannot sufficiently replicate natural conditions and diets (Ferrie *et al.*, 2014), do not know breeding cues, or the animals develop high levels of stress in captive conditions making captive husbandry an especially challenging research issue (Browne *et al.*, 2007).
3 *Becoming extinct in the wild*: The probability of avoiding extinction is linked to the probability that a species actually goes extinct in the wild. The IUCN Red List Authority listing process classifies species as near threatened, vulnerable, endangered or critically endangered, implying a differential degree of extinction risk between categories (Mace *et al.*, 2008). It is important to note, however, that red list categories are not tied to explicit extinction probabilities (Fieberg & Ellner, 2000; Mooers, Faith & Maddison, 2008). For our purposes it is necessary to quantify extinction probability and the level of uncertainty around that estimate, because incorrectly prioritizing a species not in danger of extinction could deprive a more vulnerable species of limited *ex situ* space resources and research attention.

Materials and methods

Expert selection

We identified 35 potential amphibian experts who are members of the Círculo Herpetológico de Panamá, the IUCN Amphibian Specialist Group for Panama, or who were suggested by one of the existing authors that had at least 5 years of field experience in Panama, Costa Rica or Colombia, or with equivalent captive husbandry experience with Panamanian amphibian species. We asked each expert to participate in an online questionnaire for every Panamanian amphibian species with which they had direct field or husbandry experience. We also provided information on what species were represented in captivity in Panama and at Association of Zoos and Aquariums (AZA) facilities in the United States (Table 1).

Precision and uncertainty

We devised a minimum–maximum rank weighting approach that limited responses to evenly distributed 20th percentiles as follows: very low (0–20%), low (20–40%), medium (40–60%), high (60–80%) and very high (80–100%). Where respondents had a high degree of certainty, they could also restrict the upper and lower bounds at either end of the probability range, that is 0–1% or 100%. To broaden the uncertainty bounds, respondents were allowed to select multiple categories. For each species, we asked participants to self-identify their level of experience as high, medium, low or none in both field and captive care settings so that we could weight their responses accordingly. If the participant did not know the answer to a particular question, they were asked to leave the field blank. The full survey is available in Supporting Information (Data S1) and online (available from: http://bit.ly/1oyaKPS).

Analysis

Due to recent amphibian name changes, all returns were checked for taxonomic consistency following the Amphibian Species of the World Database (Frost, 2014). As a first filter, species were ranked by the probability of observing declines when infected with *Bd*. These categories were as follows: 0 = not susceptible, declines in *Bd* positive situations will likely be undetectable; 1 = moderately susceptible, declines will be noticeable, but the species will likely persist in some places throughout its former range; 2 = highly susceptible, severe declines throughout its range and may only persist in a few places; 3 = severely susceptible, will likely disappear from entire range and will possibly go extinct without intervention. Individual answers were weighted for field experience, multiplying answers by 1 = no experience; 2 = low experience; 3 = medium experience and 4 = high experience. Totals for each species were divided by the total weighting score to calculate an average weighted susceptibility. Any species that received responses from fewer than three participants was omitted from the analysis as unknown. We focused subsequent analyses on the top quartile of most susceptible species.

The experience-weighted upper and lower probability bounds were calculated similarly for (1) establishing a viable *ex situ* population, (2) extinction in the wild in the next 20 years and (3) breeding successfully in captivity. Any

Table 1 List of Panamanian amphibian species held for conservation purposes in Panama (April 2014), the Atlanta Botanical Gardens and accredited AZA Institutions as reported in the ZIMS (April 2014)

Species	Holding facility	Male	Female	Unsexed
Agalychnis lemur	EVACC	7	1	52
	19 AZA Institutions	7	2	232
	Atlanta Botanical Garden	12	20	120
Anotheca spinosa	EVACC	49	15	80
	5 AZA institutions		1	11
	Atlanta Botanical Garden	8	6	45
Atelopus certus[a]	Gamboa ARCC	93	56	89
Atelopus glyphus[a]	Gamboa ARCC	56	45	85
	EVACC	12	6	1
Atelopus limosus[a]	Gamboa ARCC	40	17	70
	EVACC	1		13
Atelopus varius[a]	EVACC	6	10	
	3 AZA institutions	70	44	48
Atelopus zeteki[a]	EVACC	12	12	
	53 AZA institutions	498	415	359
Colostethus panamansis	EVACC	3		3
Craugastor evanesco	EVACC			35
Craugastor punctariolus	EVACC	1	2	
Craugastor tabasarae	EVACC	1	4	
Ecnomiohyla rabborum	Atlanta Botanical Garden	1		
Gastrotheca cornuta[a]	EVACC	47	26	56
	Atlanta Botanical Garden	1	1	
Hemiphractus fasciatus	EVACC	4	13	19
Hyloscirtus colymba	Gamboa ARCC		2	
Sachatamia albomaculata	Atlanta Botanical Garden	1	1	
Sachatamia ilex	EVACC	4	1	3
Strabomantis bufoniformis	EVACC	34	13	

AZA, Association of Zoos and Aquariums; ZIMS, Zoological Information Management System; ARCC, Amphibian Research and Conservation Center; EVACC, El Valle Amphibian Conservation Center.

[a]At least 10 male and 10 female founders are known to be alive or represented in the existing captive collection. *Atelopus zeteki* and *A. varius* numbers were determined from the Golden Frog Species Survival Plan report (Estrada *et al.*, 2014).

species that received responses from fewer than three participants was omitted from the analysis as unknown.

Results

Chytridiomycosis susceptibility

Twenty-three experts returned 1387 responses for 215 Panamanian amphibian species (Supporting Information Data S1). Twelve were primarily field experts, five were primarily captive husbandry experts and six experts had experience in both field and captive settings. We received at least three independent *Bd* susceptibility responses for 134 (62%) species. We present these responses as a ranked list that also acts as our first filter (Table 2). The top quartiles (34 species) were dominated by three anuran genera: *Craugastor*, *Atelopus* and *Isthmohyla* (Table 2). Higher perceived susceptibility to *Bd* scores were associated with more endangered IUCN status (Table 2), but seven species in the top quartile were not listed by the IUCN as Endangered or Critically Endangered. *Craugastor evanesco* is a recently described species that has not yet been evaluated, but belongs to the highly chytridiomycosis-susceptible *Craugastor rugulosus* species group of Central America (Ryan *et al.*, 2010). *Silverstoneia nubicola* and *Hemiphractus fasciatus* are listed as Near Threatened, whereas *Strabomantis bufoniformis*, *Anotheca spinosa*, *Colostethus panamansis* and *Hyloscirtus palmeri* are listed as Least Concern by the IUCN Red List Authority.

Finding founders

The probability of establishing founding populations was unknown for about 15% of species identified as *Bd* susceptible, and the upper probability of finding a sufficient founding population was low for 17% of *Bd*-susceptible species (Table 2). *Atelopus zeteki*, *A. certus*, *A. glyphus* and *A. limosus* were judged to have higher probabilities of successfully establishing founding populations, in part, because they are already maintained in captive colonies established prior to declines (Tables 1, 2). The overall probability of finding founding populations for the salamanders in the genus *Bolitoglossa* was also low, largely because they are rarely encountered in the field (Table 2; Supporting Information Data S2).

Experts indicated in their narrative responses that about 4% of Panama's known amphibians are missing. *Atelopus*

Table 2 Expert survey results showing Panamanian amphibian species ranked by their perceived susceptibility to *Batrachochytrium dendrobatidis* (*Bd*) and mean upper and lower estimates of factors used to determine the percentage probability of avoiding extinction through captive breeding programs

			P finding founders		*P* extinction in wild		*P* captive breeding		*P* avoiding extinction	
Species	IUCN[a]	*Bd*[b]	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Atelopus chiriquiensis	CR	**3.0**	0	1	**80**	**100**	**72**	**92**	0	1
Craugastor catalinae	CR	**3.0**					40	60		
Craugastor punctariolus	EN	**3.0**	0	1	**40**	**60**	18	42	0	1
Craugastor rhyacobatrachus	EN	**3.0**	0	1			33	60		
Craugastor taurus	CR	**3.0**	27	47			40	60		
Oophaga speciosa	EN	**3.0**					**60**	**80**		
Isthmohyla calypsa	CR	**2.9**	0	1			27	47		
Atelopus zeteki	CR	**2.8**	**81**	**86**	**72**	**96**	**64**	**83**	**37**	**68**
Ecnomiohyla rabborum	CR	**2.8**	8	17	**80**	**100**	22	34	1	5
Atelopus certus	EN	**2.7**	**84**	**98**	**57**	**84**	**70**	**92**	**33**	**76**
Atelopus glyphus	CR	**2.7**	**88**	**95**	**62**	**87**	**67**	**91**	**37**	**75**
Incilius fastidiosus	CR	**2.6**								
Isthmohyla angustilineata	CR	**2.6**					33	53		
Isthmohyla tica	CR	**2.6**	0	7			33	53		
Atelopus limosus	EN	**2.5**	**60**	**85**	**67**	**94**	**58**	**82**	**23**	**66**
Craugastor azueroensis	EN	**2.5**	38	48	**38**	**68**	40	67	**6**	**21**
Craugastor obesus	EN	**2.5**	0	7			25	45		
Isthmohyla debilis	CR	**2.5**	9	18			27	47		
Craugastor evanesco		**2.4**	33	57	**77**	**97**	27	47	**7**	**26**
Atelopus varius	CR	**2.4**	30	55	**54**	**83**	**68**	**89**	**11**	**41**
Duellmanohyla uranochroa	EN	**2.3**	37	52			27	47		
Strabomantis bufoniformis	LC	**2.3**	30	56	**41**	**76**	31	56	**4**	**24**
Isthmohyla graceae	CR	**2.3**					27	41		
Agalychnis lemur	CR	**2.2**	35	59	**37**	**65**	**61**	**83**	**7**	**32**
Anotheca spinosa	LC	**2.0**	26	60	**47**	**87**	**70**	**98**	**10**	**59**
Craugastor tabasarae	CR	**2.0**	10	44	**37**	**80**	33	62	1	22
Isthmohyla rivularis	CR	**2.0**	20	31			40	60		
Gastrotheca cornuta	EN	**2.0**	17	45	**40**	**68**	48	74	**3**	**23**
Colostethus panamansis	LC	**1.8**	**58**	**83**	**29**	**56**	34	60	**6**	**28**
Silverstoneia nubicola	NT	**1.8**					53	73		
Pipa myersi	EN	**1.8**	0	20			40	60		
Hyloscirtus colymba	CR	**1.7**	17	42	**46**	**77**	3	28	0	9
Hemiphractus fasciatus	NT	**1.7**	24	60	**45**	**79**	53	79	**6**	**37**
Hyloscirtus palmeri	LC	**1.7**	14	39	**53**	**83**	4	29	0	9
Colostethus pratti	LC	1.6	47	71	**29**	**60**	**65**	**92**	**9**	**39**
Oedipina grandis	EN	1.5	0	15			20	34		
Oophaga arborea	EN	1.5					**55**	**80**		
Oophaga granulifera	VU	1.5	**69**	**89**			**60**	**85**		
Pristimantis museosus	EN	1.5	21	48	**32**	**65**	22	54	1	17
Pristimantis moro	LC	1.4	7	33	**40**	**65**	23	43	1	9
Bolitoglossa colonnea	LC	1.4	12	27	**36**	**70**	35	55	2	10
Craugastor underwoodi	LC	1.4	40	68	**31**	**57**	33	53	**4**	**21**
Craugastor rugosus	LC	1.3	13	44			33	53		
Craugastor opimus	LC	1.3					34	60		
Hyalinobatrachium aureoguttatum	NT	1.3			27	53	53	73		
Cochranella euknemos	LC	1.2	15	34	**30**	**58**	33	55	2	11
Craugastor megacephalus	LC	1.2	23	48	**31**	**57**	35	57	2	16
Bolitoglossa schizodactyla	LC	1.2	15	33			27	47		
Phyllobates lugubris	LC	1.1	52	72	24	49	50	73	**6**	**25**
Hyalinobatrachium colymbiphyllum	LC	1.1	58	77	5	36	44	64	1	18
Silverstoneia flotator	LC	1.1	**73**	**99**	4	33	53	80	**2**	**26**
Craugastor monnichorum	DD	1.1	22	42	**38**	**62**	32	56	3	15
Sachatamia ilex	LC	1.1	30	59	25	53	36	60	3	19

Table 2 Continued.

Species	IUCN[a]	*Bd*[b]	*P* finding founders		*P* extinction in wild		*P* captive breeding		*P* avoiding extinction	
			Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Lithobates warszewitschii	LC	1.1	47	71	26	51	25	47	3	17
Craugastor gollmeri	LC	1.1	23	47	27	53	38	58	2	14
Pristimantis caryophyllaceus	NT	1.0	45	66	18	39	24	46	2	12
Craugastor noblei	LC	1.0	9	37	20	54	40	60	1	12
Isthmohyla lancasteri	LC	1.0	40	60			40	60		
Andinobates claudiae	DD	1.0								
Pristimantis gaigei	LC	1.0	14	40	**29**	**53**	49	70	2	15
Craugastor talamancae	LC	1.0	40	64	11	39	40	64	2	16
Pristimantis pardalis	NT	0.9	21	46	**31**	**52**	30	52	2	12
Ctenophryne aterrima	LC	0.9	3	8			30	45		
Incilius aucoinae	LC	0.9	**87**	**100**	10	30				
Dendrobates auratus	LC	0.9	**73**	**93**	10	35	**70**	**94**	**5**	**30**
Hyalinobatrachium talamancae	LC	0.9	46	75	**33**	**58**	36	68	**6**	**30**
Pristimantis taeniatus	LC	0.9	38	64	24	51	40	68	**4**	**22**
Hyalinobatrachium chirripoi	LC	0.9	20	45	14	42	37	57	1	11
Agalychnis spurrelli	LC	0.8	44	67	24	44	**75**	**95**	**8**	**28**
Hyalinobatrachium valerioi	LC	0.8	17	40	**33**	**60**	**64**	**84**	**4**	**20**
Incilius coniferus	LC	0.8	57	78	15	36	**59**	**84**	**5**	**24**
Bolitoglossa medemi	VU	0.8								
Teratohyla pulverata	LC	0.8	21	42	16	43	35	55	1	10
Andinobates minutus	LC	0.8	**65**	**92**	15	44	51	71	**5**	**28**
Isthmohyla zeteki	NT	0.8	22	40	**37**	**57**	40	60	3	14
Pristimantis cruentus	LC	0.8	34	67	17	44	30	57	2	17
Teratohyla spinosa	LC	0.8	48	73	16	39	52	72	**4**	**20**
Pristimantis cerasinus	LC	0.7	50	75	15	41	28	48	2	15
Hypsiboas rufitelus	LC	0.7	**60**	**81**	20	45	29	51	3	19
Pristimantis ridens	LC	0.7	52	73	13	33	30	50	2	12
Craugastor melanostictus	LC	0.7	40	61	20	50	40	60	3	18
Cruziohyla calcarifer	LC	0.7	5	20			**66**	**86**		
Allobates talamancae	LC	0.7	**65**	**86**	9	25	**64**	**87**	4	19
Incilius signifer	LC	0.7					**56**	**80**		
Oophaga vicentei	DD	0.7	**80**	**94**	19	46	55	75	**8**	**32**
Espadarana prosoblepon	LC	0.6	**69**	**90**	12	29	46	70	4	19
Sachatamia albomaculata	LC	0.6	**59**	**82**	11	35	50	72	**3**	**21**
Craugastor podiciferus	NT	0.6	**60**	**88**	**33**	**63**	33	60	**7**	**33**
Bolitoglossa compacta	EN	0.6	0	7			20	34		
Oedipina complex	LC	0.6	7	27			20	37		
Ptychohyla legleri	EN	0.6					27	40		
Craugastor bransfordii	LC	0.6	45	68	14	39	40	63	3	16
Smilisca sordida	LC	0.6	56	76	20	47	53	73	**6**	**26**
Hyalinobatrachium fleischmanni	LC	0.5	56	78	9	31	**58**	**82**	3	20
Rhaebo haemititicus	LC	0.5	58	80	12	35	52	77	**4**	**21**
Dendropsophus ebraccatus	LC	0.4	**70**	**90**	7	22	**73**	**93**	3	19
Craugastor crassidigitus	LC	0.4	**73**	**93**	7	31	50	73	**2**	**21**
Cochranella granulosa	LC	0.4	50	73	12	35	**68**	**88**	**4**	**23**
Diasporus diastema	LC	0.4	**58**	**82**	8	25	27	50	1	10
Bolitoglossa marmorea	EN	0.4					13	27		
Phyllomedusa venusta	LC	0.4	27	60			38	70		
Bolitoglossa lignicolor	VU	0.4	12	24						
Bolitoglossa minutula	EN	0.3								
Diasporus quidditus	LC	0.3	**67**	**90**	11	38	33	57	2	19
Leptodactylus savagei	LC	0.3	53	76	17	37	35	70	3	20
Oedipina parvipes	LC	0.3					33	40		
Smilisca sila	LC	0.3	**60**	**83**			**53**	**80**		
Craugastor fitzingeri	LC	0.3	**67**	**88**	6	21	**57**	**80**	2	15

Table 2 Continued.

Species	IUCN[a]	*Bd*[b]	*P* finding founders		*P* extinction in wild		*P* captive breeding		*P* avoiding extinction	
			Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Rhinella alata		0.3	**70**	**89**	6	26	**60**	**80**	3	18
Smilisca phaeota	LC	0.3	**57**	**84**	5	29	51	76	2	18
Chiasmocleis panamensis	LC	0.3	40	73	20	45	33	53	3	18
Rhinella centralis	LC	0.2	**68**	**96**	0	5	**60**	**80**	0	4
Lithobates vaillanti	LC	0.2	59	79	**40**	**60**	48	68	**11**	**32**
Agalychnis callidryas	LC	0.2	**76**	**98**	4	22	**77**	**100**	**3**	**21**
Engystomops pustulosus	LC	0.2	**74**	**100**	3	21	**67**	**93**	2	19
Dendropsophus microcephalus	LC	0.2	**75**	**97**	3	12	**67**	**87**	1	10
Crepidophryne epiotica	LC	0.1	36	58	14	31				
Leptodactylus melanonotus	LC	0.1	**60**	**80**	11	31	40	60	3	15
Craugastor stejnegerianus	LC	0.1	**75**	**95**	5	28	33	60	1	16
Bolitoglossa biseriata	LC	0.0					20	40		
Bolitoglossa magnifica	EN	0.0								
Dendropsophus phlebodes	LC	0.0	**76**	**96**	5	12	**70**	**90**	3	10
Diasporus hylaeformis	LC	0.0	40	67	18	40	33	53	2	14
Hypsiboas boans	LC	0.0					47	73		
Hypsiboas pugnax	LC	0.0					47	73		
Hypsiboas rosenbergi	LC	0.0	55	83	12	37	33	57	2	17
Isthmohyla picadoi	NT	0.0	18	44			40	60		
Leptodactylus bolivianus	LC	0.0	45	64	8	28	40	60	1	11
Leptodactylus fragilis	LC	0.0	**60**	**80**	6	12	40	60	1	6
Oophaga pumilio	LC	0.0	**76**	**100**	5	25	**65**	**90**	**3**	**23**
Pleurodema brachyops	LC	0.0	**76**	**96**	0	1	55	75	0	1
Rhinella marina	LC	0.0	**78**	**97**	0	3	**77**	**97**	0	3
Scinax altae	LC	0.0					53	73		
Scinax boulengeri	LC	0.0	45	69	12	40	53	73	**3**	**20**
Scinax elaeochrous	LC	0.0	**65**	**85**			**53**	**80**		
Scinax ruber	LC	0.0	40	80	20	47	53	73	**4**	**28**
Trachycephalus typhonius	LC	0.0	40	65	20	40	**53**	**80**	**4**	**21**

Bold numbers indicate the species was ranked in the top quartile of responses. Blanks indicate that fewer than three expert responses were received. The 78 species for which fewer than three responses were received evaluating their *Bd* susceptibility are not included in this table but responses may be viewed in Supporting Information (Data S2).

[a]IUCN Red List status are indicated in the first column.

[b]Perceived susceptibility to *Bd* is indicated in the second column [ranging from 3 (most susceptible) to 0 (least susceptible)].

chiriquiensis, *Craugastor emcelae*, *C. obesus*, *C. punctariolus*, *C. rhyacobatrachus*, *Ecnomiohyla rabborum*, *Isthmohyla calypsa* and *Oophaga speciosa* have not been observed for 8–15 years. Therefore, the likelihood of finding sufficient numbers for captive assurance populations for these species is exceedingly low (Table 2; Supporting Information Data S1). The 78 amphibian species for which there were insufficient responses to assess *Bd* susceptibility are likely also rare; ~40% of these were salamanders or caecilians (Supporting Information Data S2). The absence of confidence by the experts to provide a formal assessment also indicates a likely inability to establish sufficient founder populations of these species.

Probability of extinction in wild

Perceived *Bd* susceptibility was strongly correlated with the probability that the species would go extinct in the wild in the next 20 years [Pearson's r (91) = 0.86 $P < 0.001$). All of the species perceived to be the most sensitive to *Bd* were within the top quartile of species at the highest risk of extinction (Table 2). Higher expert evaluations of extinction risk in the next 20 years were strongly associated with more threatened IUCN risk categories (Table 2).

Captive breeding

Of the most chytridiomycosis-susceptible species, frogs of the genera *Atelopus*, *Oophaga*, *Agalychnis* and *Anotheca* had the best chances of establishing viable, long-term captive populations, whereas frogs of the genus *Hyloscirtus* were the least likely to succeed under *ex situ* conditions (Table 2). There was no apparent relationship between the likelihood of success with captive breeding and extinction probability or the likelihood of finding founding populations (Table 2).

Probability of avoiding extinction through captive breeding

We had sufficient expert responses in all categories for 19 of the 36 species most susceptible to *Bd* (Fig. 1). Of these, the four highest ranked species were *Atelopus glyphus*, *A. certus*, *A. limosus* and *A. zeteki*, with a medium-to-high chance of avoiding extinction through captive breeding colonies. *Craugastor evanesco*, *C. tabasarae*, *C. azueroensis*, *Strabomantis bufoniformis*, *Colostethus panamansis*, *Gastrotheca cornuta*, *Agalychnis lemur*, *Hemiphractus fasciatus*, *Atelopus varius* and *Anotheca spinosa* had very low-to-medium chances of avoiding extinction through captive breeding due to a combination of difficulty finding founders and/or ability to breed them in captivity (Fig. 1; Table 2). At the lowest end of the scale were *Craugastor punctariolus*, *Atelopus chiriquiensis* and *Ecnomiohyla rabborum*. For these species, the chance of avoiding extinction through captive breeding was very low, primarily because they have already disappeared. The two *Hyloscirtus* species were ranked low primarily because of challenges associated with *ex situ* care and breeding (Table 2; Fig. 1). The prognosis of success for the other 17 species perceived to be highly susceptible to *Bd* was projected to be low, largely due to the challenges of establishing a founding population (Table 2).

Discussion

Chytridiomycosis susceptibility

There was a close association between chytridiomycosis susceptibility scores and IUCN status that was not surprising because chytridiomycosis was a major threat considered during the last systematic IUCN red list conservation assessment of Panamanian species in 2008. Of more interest were species perceived to be highly susceptible to chytridiomycosis that are not currently listed in threatened IUCN categories such as *Craugastor evanesco*, *Strabomantis bufoniformis*, *Anotheca spinosa*, *Colostethus panamansis* and *Hyloscirtus palmeri*. This should be justification for immediate assessment or reassessment.

More than half of the species that experts considered most sensitive to chytridiomycosis belonged to the genera *Atelopus*, *Isthmohyla* and *Craugastor*. All six *Atelopus* species were ranked in the top quartile of most *Bd*-sensitive species. This genus has experienced widespread declines throughout the neotropics, and >30 of 97 species are now feared extinct, primarily because of *Bd*-related declines (La Marca *et al.*, 2005). *Atelopus* are charismatic, diurnal toads that normally exist at high densities, so their absences have been particularly noticeable compared to other more cryptic or rare species (Lindquist & Swihart, 1997; La Marca *et al.*, 2005). The hypersensitivity of *Atelopus* to chytridiomycosis has been demonstrated in both laboratory and field settings that validate this assessment (Woodhams *et al.*, 2006; Bustamante, Livo & Carey, 2010; Richards-Zawacki, 2010; Becker *et al.*, 2012; Langhammer *et al.*, 2013).

Stream-dwelling frogs of the *Craugastor punctariolus* species series (Campbell & Savage, 2000) have experienced significant *Bd*-related declines throughout much of their range (Mason, Karen & Michael, 2008; Ryan *et al.*, 2010; Zumbado-Ulate *et al.*, 2011). Panama has eight species in this species series: *C. azueroensis*, *C. catalinae*, *C. evanesco*, *C. obesus*, *C. punctariolus*, *C. ranoides*, *C. rhyacobatrachus*, *C. taurus* (Campbell & Savage, 2000; Ryan *et al.*, 2010); all were highly *Bd* sensitive with at least one species having poor innate skin defenses (Woodhams *et al.*, 2006).

All of the *Isthmohyla* identified by our experts as most susceptible to chytridiomycosis are listed by the IUCN as critically endangered. Disappearances of *Isthmohyla*, such as *I. rivularis*, *I. angustilineata*, *I. tica*, *I. calypsa*, have been attributed to chytridiomycosis (Lips, 1999; Cheng *et al.*, 2011; Garcia-Rodriguez *et al.*, 2012), and our experts placed these in the top quartile of the most sensitive species. Other

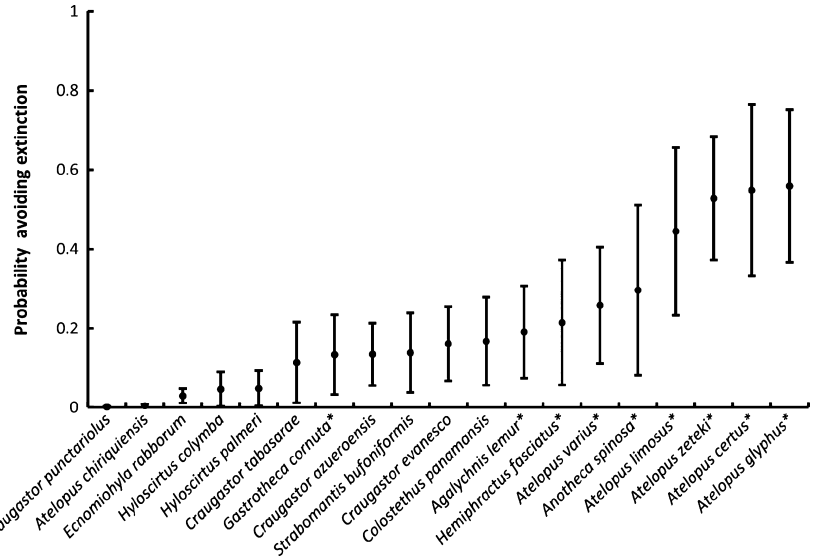

Figure 1 Ranked probability of avoiding extinction through captive breeding for Panamanian amphibian species perceived to be most susceptible to *Batrachochytrium dendrobatidis* (*Bd*). Shown are the range between mean lower and mean upper probability estimates derived through an expert assessment. Asterisk (*) indicates species has been maintained and bred reliably in captive conditions, and species for which we did not receive sufficient responses are not shown.

Isthmohyla species, such as bromeliad-dwelling *I. picadoi* and *I. zeteki* and pond-breeding *I. pseudopuma*, persist in *Bd*-positive areas (Stuckert *et al.*, 2009) and were considered less susceptible (Table 2).

Experts are powerful integrators of information and can accommodate differences between species' natural history that would be difficult to capture any other way. It is reassuring to find that existing literature corroborated this assessment, but self-reinforcement or erroneous thinking is an inherent risk of using experiential knowledge in small communities familiar with each other's work (Fazey *et al.*, 2006). Expert assessments require checks and balances in the form of empirical evidence (Fazey *et al.*, 2006). Controlled exposure experiments (Blaustein *et al.*, 2005; Martel *et al.*, 2014; Voyles *et al.*, 2014) and mucosome tests (Woodhams *et al.*, 2014) would be powerful validation tools that could help verify this study's assessment of chytridiomycosis sensitivity more extensively than covered here.

Rare and 'lost' species

Rare species of amphibians often have restricted ranges and small population sizes, which are both factors increasing vulnerability to extinction (Toledo *et al.*, 2014). While the conservation needs within this category are likely to be high, our lack of basic natural history knowledge and the inability to find many of these animals in the wild currently limits captive breeding as a potential conservation tool. Because of a dearth of information, there is a need for more natural history field studies, especially of tropical salamanders and caecilians.

For species that were once more abundant but have disappeared, rediscovery is a critical next step. Our experts identified *Atelopus chiriquiensis*, *A. zeteki*, *Craugastor emcelae*, *C. obesus*, *C. punctariolus*, *C. rhyacobatrachus*, *Ecnomiohyla rabborum*, *Isthmohyla calypsa* and *Oophaga speciosa* as 'Panama's lost frogs'. In the case of *O. speciosa*, at least 31 were exported from Panama since being listed by CITES in 1987 (Carpenter *et al.*, 2014), but it is unclear whether any descendants of that population persist in captivity. *Ecnomiohyla rabborum* is down to one last remaining captive individual held at the Atlanta Botanical Gardens (M. Mandica, pers. comm.). A few individuals presumed to be *C. punctariolus* remain at EVACC, but it is a species that is difficult to identify as it is part of cryptic species complex (Crawford *et al.*, 2013). Over the course of 7 years EVACC has produced several clutches of mostly infertile eggs and has been unable to rear young to adulthood (Supporting Information Data S2). All of these 'lost' frog species are possibly extinct in nature and only *Atelopus zeteki* is now secure in captivity in the USA and Panama.

Missing species that have been rediscovered in the wild are termed 'Lazarus' species, but what subsequent actions are needed remain open for debate (Meijaard & Nijman, 2014). Several neotropical frog species that were absent for protracted intervals, such as *Incilius holdridgei*, *Atelopus varius*, *Lithobates vibicarius*, *Craugastor taurus*, have been found again, but at low densities (Kubicki, 2004, 2006; Abarca *et al.*, 2010; Garcia-Rodriguez *et al.*, 2012; Hertz *et al.*, 2012; Delia, Whitney & Burkhardt, 2013; Chaves *et al.*, 2014). In these cases there are too few animals to collect a founding population (Frankham *et al.*, 2002), and supplementing small wild populations with captive-bred animals has its own risks (Jensen, 2013). Intensive study of these populations and monitoring of threats would be recommended in order to develop a clear information-based conservation strategy.

Captive experience

The probability of breeding an animal in captivity for about two thirds of Panama's species fell in the medium to high range, indicating that experts were confident that husbandry challenges could be solved for a majority of the species. One of the lessons learned as a byproduct of this epidemic is that general husbandry approaches may work for the majority of species, but simply collecting a full founding population and exerting substantial effort on husbandry approaches may not work for every species. For example, founding populations were collected and maintained for *H. colymba* and *C. punctariolus*, and despite successful but limited egg laying, we were unable to rear offspring through metamorphosis (Supporting Information Data S2).

Another key factor is having enough time to resolve the limiting elements to effective husbandry, nutrition and reproduction. For example, the Panamanian Golden Frog Husbandry Manual (Poole, 2006) captured 10 years of experience and trial and error with golden frogs in captivity. These protocols have, in turn, benefited other Panamanian *Atelopus* species including *A. certus*, *A. glyphus*, *A. limosus* and *A. varius* (R. Ibáñez, pers. comm.). Such knowledge also has increased the confidence by experts in the success of future captive breeding for *Agalychnis lemur*, *Hemiphractus fasciatus*, *Gastrotheca cornuta* and *Anotheca spinosa*, all of which had high rankings in the survey for likely avoiding extinction, in part due to a successful captive breeding track record. It is clear from these examples that the information learned from captive breeding programs is an important factor that should be incorporated into the prioritization process despite the fact that the lessons learned are mostly qualitative and seldom published.

Recommendations

Less-susceptible species

About 100 species, about half of Panama's amphibian species can still be reliably found, even in *Bd*-positive areas and have not experienced severe chytridiomycosis declines (Table 2). We do not recommend establishing and maintaining *ex situ* assurance populations for these species unless non-*Bd*-related threats are a pressing concern, or new evidence is found demonstrating their susceptibility to chytridiomycosis.

Lost frogs and rare species

Our study identified 81 species, where not enough information was known to provide an evaluation, and nine species that were once fairly common but have disappeared (Sup-

porting Information Data S2). Most of the species that we have limited information for are likely to be rare species, and we know that rare species or those with very limited distributions are more likely to be in danger of extinction (Toledo *et al.*, 2014). In both cases, efforts should be made to survey known localities of these species and obtain more reliable information on their status, particularly if their congeners are *Bd* susceptible. Genomic material should be banked for these species including living cell lines, living gametes and cryopreserved tissues (Lermen *et al.*, 2009; Kouba *et al.*, 2013).

Bd-sensitive species that have declined severely or are likely to decline severely

Craugastor azueroensis is not represented in current captive breeding collections, but they are extant members of the *Craugastor punctariolus* species series that can still be found reliably in the wild (Köhler *et al.*, 2012). This species is a potential candidate for captive breeding efforts even though the combined probability of avoiding extinction is low, the chances of avoiding an extinction through captive breeding are better than for several of the other species. Studying wild populations and learning more about their natural history, improving and resolving captive husbandry approaches, exploring assisted reproduction methods and collecting wild founding populations while these species are still extant will help improve the odds of success. A captive breeding population represented by at least 10 males and 10 females exists in Panama for *Atelopus glyphus*, *A. certus* and *A. limosus*, and these species are not held elsewhere in captivity (Table 1). Existing unrepresented founders should be bred as soon as possible, capturing as much of their genetic diversity as possible, with a goal of ensuring even representation of founder's genes in a total captive population of 500 individuals (Schad, 2008). A captive population represented by at least 10 males and 10 females exists for *Atelopus zeteki* and *Atelopus varius* in the USA and Panama (Table 1), and captive management steps are already being taken to coordinate the population management internationally as part of the AZA Golden Frog Species Survival Plan (Estrada *et al.*, 2014). Similarly, a captive population represented by at least 10 males and 10 females exists for *Gastrotheca cornuta* in Panama and the USA, but a formal Species Survival Plan or animal exchange plan does not exist between the two holding institutions and should be explored.

Partial founding populations of *Hemiphractus fasciatus*, *Craugastor evanesco*, *Colostethus panamansis* and *Strabomantis bufoniformis* exist only in Panama. *Hemiphractus fasciatus* has been bred in captivity, but additional expeditions to collect additional founders are needed. *Strabomantis bufoniformis* has been bred successfully, but not reliably in captivity, while *C. evanesco* and *C. panamansis* have not been bred in captivity (Supporting Information Data S2). Focused work on developing husbandry protocols and identifying breeding cues or researching assisted reproduction methods for these species is needed.

Partial founding populations exist in Panama and elsewhere for *Anotheca spinosa* and *Agalychnis lemur* (Table 1). These species have all been reliably bred in captivity, but international coordination and cooperation to manage holdings in multiple institutions is required to ensure that the genetic integrity of the captive population as a whole is maintained. Targeted collecting expeditions may be needed to collect additional founders if the combined captive population is not represented by adequate founders.

At present we do not have an ability to reverse the threat of *Bd* in the wild, so a more holistic conservation prioritization scheme that aims to get the best return on investment considering multiple threats and taxa might logically focus resources on other more tractable problems and species. Captive breeding alone does not solve the threat of chytridiomycosis, but it does buy us the time needed to continue the incremental research that may help tip the scales in favor of the frogs, and given our strong track record of solving disease problems in humans and domesticated species, we are hopeful that we will eventually be able to solve this critical wildlife disease threat. Only healthy assurance populations will help us avoid extinctions of highly susceptible species in the short term and provide the foundation for reintroductions over the longer term. This assessment has helped us to evaluate the likelihood that we will be able to avoid extinctions and to focus our resources appropriately in the longer term.

Acknowledgments

We thank Ron Gagliardo for sharing information on captive breeding efforts, David Wildt and two anonymous reviewers who provided helpful comments on this manuscript. The PARC project is a partnership between the Houston Zoo, the Zoo New England, the Cheyenne Mountain Zoo, the Smithsonian Conservation Biology Institute and the Smithsonian Tropical Research Institute. Grants from Minera Panama, Banco Bilbao Vizcaya Argentaria and USAID supported the contributions of PARC employees BG, HR, JG and RI. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Data S1. Survey questions: evaluating the probability of avoiding disease-related extinctions of Panamanian amphibians through captive breeding programs.

Data S2. All survey responses collected to evaluate the probability of avoiding disease-related extinctions of Panamanian amphibians through captive breeding programs.

Anexo 2. Permiso científico de investigación y colecta del proyecto PARC (SE/AP-34-15), emitido por el Ministerio de Ambiente.

REPUBLICA DE PANAMA	Autoridad Nacional del Ambiente	PERMISO CIENTÍFICO SCIENTIFIC PERMIT
	MINISTERIO DE AMBIENTE Apartado C, Zona 0843, Balboa, Ancón, Panamá	No. SE/AP-34-15 VALIDO HASTA: 31-12-2016 VALID UNTIL
		FAUNA / FLORA / HONGOS / BACTERIAS / OTROS ORGANISMOS **FAUNA,FLORA**

INVESTIGADOR RESPONSABLE MAIN RESEARCHER	IDENTIFICATION I.D.	COLECTA / OBSERVACION COLLECT / OBSERVATION MARCADO / OTROS MARKING / OTHERS
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INSTITUCION QUE RESPALDA LA INVESTIGACION
INSTITUTE SUPPORTING THE RESEARCH
INSTITUTO DE INVESTIGACIONES TROPICALES SMITHSONIAN

OBJETIVO
PURPOSE
*Continuar estudios de sistemática de anfibios de los anfibios de Panamá
. *Realizar observaciones sobre la ecología y el comportamiento de los anfibios de Panamá.
*Determinar el patrón de distribución, dispersión y persistencia del hongo quitridio que afecta a las poblaciones de anfibios.
*Obtener información acerca de la dieta de los anfibios amenazados.
*Determinar la presencia de las toxinas en la piel de los anfibios.
*Establecer la existrencia de hospederos y vectores no-anfibios asociados al hongo quitridio.
*Desarrollar un programa de conservación ex situ de anfibios.

LUGAR DEL ESTUDIO
STUDY
P.N. Chagres, P.N. Darién, P.N. GDOTH (COPE), P.N Soberanía, P.N. Altos de Campana, A.P. Donoso, R.F. La Tronosa, P.N. cerro Hoya, P.I. La Amistad, P.N. Santa Fe, B.P. Alto Darién, R.H. filo del Tallo, P.P. San Lorenzo, P.N. Portobelo,A.U.M. Donoso.

Provincias de Bocas del Toro, Chiriquí, Colon, Coclé, Darién, Herrera, Los Santos, Panamá, panamá Oeste. Y Veraguas.

****No incluye áreas que no cuenten con consentimiento libre informado previo, aprobados por la Autoridad Nacional del Ambiente****

NOMBRE COMUN COMMON NAME	NOMBRE CIENTÍFICO SCIENTIFIC NAME	CANTIDAD QUANTITY	DESCRIPCIÓN DESCRIPTION
VER LISTA ADJUNTA			

DURACION DEL ESTUDIO
TIME OF STUDY
01-01-2016 Hasta 31-12-2016

STRI WARNS YOU OF THE FOLLOWING: Under no circumstances, should samples collected under the terms and conditions of this permit be used in research that pursues economical or commercial benefits of any kind, or for any other goal not academic in nature or not oriented towards the increase of basic scientific knowledge.

ESTE PERMISO ES EMITIDO POR:
THIS PERMIT IS ISSUED BY:

Dirección de
Áreas Protegidas
y Vida Silvestre

FECHA:
DATE 01-12-2015

LIC. JORGE GARCIA
Director encargado de Áreas Protegidas y Vida Silvestre

FIRMA RESPONSABLE Y SELLO

Elaborado por:

Darío A. Luque
VoBo jefe DBVS

TODO INVERSTIGADOR DEBE: 1. REPORTARSE A LA REGION CORRESPONDIENTE ANTES DE INICIAR SU TRABAJO; 2. PORTAR EN TODO MOMENTO EL PERMISO CORRESPONDIENTE; 3. ENVIAR INFORME DE COLECTA A LA ANAM; 4. ENTREGAR MUESTRAS DE CADA ESPECIE A LA UNIVERSIDAD DE PANAMA; 5. ENVIAR A LA ANAM, A MAS TARDAR EN UN AÑO, AVANCE O INFORME FINAL DEL ESTUDIO, CON RESUMEN EN ESPAÑOL DE LO CONTRARIO NO SE LE OTORGARA OTRO PERMISO.

EVERY RESEARCHER MUST: 1. REPORT TO THE NEAREST ANAM OFFICE BEFORE START WORKING; 2. CARRY THE PERMIT AT ALL TIMES; 2. SEND A REPORT OF COLLECTED MATERIAL TO ANAM; 4. SEND SAMPLES OF REPRESENTATIVE MATERIAL TO THE UNIVERSITY OF PANAMA; 5. SEND AN INTERMEDIATE OR FINAL REPORT TO ANAM WITHIN A YEAR, WITH A SPANISH SUMMARY.
NO MORE PERMITS WILL BE ISSUED TO THOSE WHO DO NOT COMPLY WITH THESE REGULATIONS.

IMPORTANT

MINISTERIO DE AMBIENTE

MINISTERIO DE AMBIENTE
DIRECCION DE ÁREAS PROTEGIDAS Y VIDA SILVESTRE

Continuación del listado de especies a colectar y asistentes a participar del permiso **SE/AP-34-15** a nombre de Roberto Ibáñez Díaz. El permiso es válido hasta el 31 de Diciembre de 2016 y es respaldado por el Instituto Smithsonian de Investigaciones Tropicales.

Lista de asistentes:

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Matthew Evans.	486562517-U.S.A.

---------- FIN DEL LISTADO---------

Listado de especies a colectar

NOMBRE COMUN COMMON NAME	NOMBRE CIENTÍFICO SCIENTIFIC NAME	CANTIDAD QUANTITY	DESCRIPCIÓN DESCRIPTION
Anfibios(para programde conservacion ex situ)	*Amphibia spp.*	54 individuo	Tamaño variable longitud <20 cm
Anfibios	*Amphibia spp.*	20 renacuajos por especie.	Tamaño variable longitud < 5 cm
Vertebrados	*Vertebrata Spp.*	3000 frotis de piel	
Artropodos	*Arthripoda spp.*	Máximo 300 muestras por sitio	Tamaño variable longitud <8 cm
Algas	*Algae y Cianobacteria spp.*	1 muestra por sitio	5 gramos

---------- FIN DEL LISTADO---------

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